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AUTOIMMUNE HEPATITIS

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ABSTRACT

Autoimmune hepatitis (AIH) is a severe hepatopathy characterised by female preponderance, hypertransaminasaemia, elevated levels of immunoglobulin (Ig) G, presence of serum autoantibodies and, histologically, by interface hepatitis. AIH occurs both in adults and children, being particularly aggressive in the latter. According to the type of serum autoantibodies, AIH can be differentiated in two forms: one positive for smooth muscle antibody (SMA) and/or antinuclear antibody (ANA) (type 1 AIH, AIH-1) and another positive for liver kidney microsomal antibody type 1 (LKM-1) (type 2 AIH, AIH-2). These two forms differ with regard to age at onset (earlier in the case of AIH-2), mode of presentation (fulminant hepatic failure more frequently observed in AIH-2) and association with IgA deficiency (more frequent in AIH-2). AIH responds satisfactorily to immunosuppressive treatment (corticosteroids with or without azathioprine) that should be started as soon as the diagnosis is made. Despite immune suppression, some 40% of patients experience relapse and 9% undergo liver transplantation.

Though the exact mechanism leading to loss of immune-tolerance in AIH is still unclear, recent evidence has pointed to a numerical and functional defect of CD4^{pos}CD25^{pos} regulatory T-cells as a factor permitting autoaggressive CD4 and CD8 T-cells to react against liver autoantigens. The generation and expansion of regulatory T-cells with liver autoantigen specificity *in vitro* represents a potential immunotherapeutic tool for the reconstitution of immune-tolerance in AIH without the drawback of pan-immunosuppression.

INTRODUCTION

Autoimmune hepatitis (AIH) is an inflammatory liver disorder characterised by female preponderance, elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), immunoglobulin G (IgG), serum autoantibodies and histologically by interface hepatitis [1]. AIH responds satisfactorily to immunosuppression that should be started promptly after diagnosis. If left untreated, AIH usually progresses to end-stage liver disease requiring transplantation [2]. According to the nature of the autoantibodies detected at diagnosis, two forms of AIH can be distinguished: type-1 AIH (AIH-1), characterised by seropositivity for smooth muscle antibody (SMA) and/or anti-nuclear antibody (ANA); and type 2 AIH, seropositive for liver kidney microsomal type 1 (LKM-1) and for liver cytosol type 1 (LC1) antibodies. A third subgroup of AIH, characterised by the presence of antibodies to a soluble liver antigen (anti-SLA) in the absence of conventional autoantibodies and referred to as AIH type 3, has been proposed [3]; subsequent studies have, however, shown the presence of anti-SLA autoantibodies in patients positive for ANA/SMA or LKM-1. Moreover no clinical features distinguish this type of autoimmune hepatitis from those previously mentioned, although the presence of anti-SLA antibodies defines a group of patients with a more severe clinical course [4]. AIH-1 affects both adults and children, while AIH-2 is mainly a paediatric disease. AIH-1 and AIH-2 respond similarly to immunosuppression, but the two forms differ with regard to age at onset, mode of presentation, successful treatment withdrawal and molecular targets of autoimmune attack. Paediatric AIH, which is the theme of the present review, has a particularly aggressive course.

EPIDEMIOLOGY AND CLINICAL FEATURES

AIH occurs worldwide across all age groups. Its reported prevalence ranges from 1.9 cases per 100,000 in Norway [5], 1/200,000 in the US general population [6] to 20/100,000 females over 14 years of age in Spain [7]. A recent study from the United Kingdom, conducted in a secondary care referral centre, has shown that the annual incidence of AIH is of 3.5/100,000 inhabitants [8]. The disease is considerably less frequent in Japan, where the incidence is between 0.34 and 0.42 cases per 100,000 people per year [9], and it accounts for 5-10% of paediatric liver disease in Brazil [10]. These figures, however, are probably underestimates as AIH may remain undiagnosed for several years and finally present with decompensated liver disease. AIH-2 prevalence is still unknown, mainly due to the fact that the diagnosis is often overlooked.

In 50% of the cases the onset is insidious and often associated with lethargy, malaise, arthralgia and myalgia; between 30 to 40% of patients present with an acute hepatitis, characterised by jaundice, dark urine and pale stools; the remaining 10-20% are incidentally discovered to have elevated transaminase levels on biochemical screening [11]. Occasionally the first symptoms of AIH are complications of portal hypertension, such as gastrointestinal bleeding or hypersplenism without previous knowledge of liver disease. An acute presentation is more frequent in children and young adults than later on in life. At times the disease presents with fulminant hepatic failure, particularly in the case of AIH-2. AIH can be associated with other autoimmune disorders such as nephrotic syndrome, thyroiditis, Behçet's disease, ulcerative colitis, insulin dependent diabetes mellitus, hypoparathyroidism and Addison's disease [12]. Prevalence of autoimmune disorders in first degree relatives is documented in 40% of cases [12, 13]. The duration of symptoms before presentation, the frequency of hepatosplenomegaly and the severity of portal tract inflammation at diagnosis are similar in the two forms of AIH. However, studies from two European Paediatric series

[12, 14] have shown that despite a similar clinical course, AIH-2 presents at a younger age and more frequently with fulminant hepatic failure than AIH-1 and it is also more frequently associated with IgA deficiency [12, 14].

A recent study from Grammatikopoulos et al has reported that high immunoglobulin G subclass 4 (IgG4) levels, which have been associated to liver disease in adults, are present in some 30% of children with AIH. High IgG4 do not correlate with biochemical and histological indices of disease activity, and do not characterise a specific disease subtype [15].

An ANA/SMA overlap syndrome between AIH and sclerosing cholangitis, diagnosed on the basis of characteristic bile duct changes on cholangiography, is observed in young patients and is referred to as autoimmune sclerosing cholangitis (ASC) [12, 16]. Compared to primary sclerosing cholangitis, mainly present in male adults and characterised by advanced fibroinflammatory damage of the intra and extra-hepatic bile ducts, ASC affects equally males and females, presents with less advanced bile duct lesions, has clinical, biochemical, immunological and histological features indistinguishable from those of AIH-1 and responds to immunosuppressive treatment [16].

DIAGNOSIS

The diagnosis of AIH is based on a combination of clinical, biochemical, immunological and histological features. Interface hepatitis is the histological hallmark of AIH and consists of a dense lymphoplasmacytic infiltrate of the portal tracts crossing the limiting plate and invading the surrounding parenchyma (Figure 1). Other typical findings are represented by hepatocyte swelling and/or piknotic necrosis, panlobular hepatitis with bridging necrosis in the acute presentation and in case of acute liver failure by massive necrosis and multilobular collapse.

Determination by immunofluorescence of serum autoantibodies not only assists in AIH diagnosis but also aids to differentiate the two forms of the disease. Routine testing for autoantibodies is performed by indirect immunofluorescence on a freshly prepared rodent substrate, including kidney, liver and stomach tissues, to allow the detection of ANA, SMA anti-LKM-1 and anti-LC1. Guidelines provided by the International Autoimmune Hepatitis Group [17, 18] have established that in adults the autoantibody positivity cut-off titre is 1/40. However, since autoantibodies are rare in children ANA and SMA titres as low as 1/20 or anti-LKM-1 titres as low as 1/10 are significant [2, 19].

In AIH ANA typically gives a homogeneous staining pattern on Hep2 cells; these cells, however, derived from a laryngeal carcinoma, are not the correct substrate for screening purposes, because of a high proportion of low titre positivity in healthy people. ANA are a heterogeneous group of autoantibodies reacting with a broad spectrum of nuclear components such as single- and double-stranded deoxyribonucleic acid (DNA), small nuclear ribonucleoproteins (snRNPs), centromere, lamin, histones, chromatin and cyclin A. The mechanism leading to the production of ANA in AIH is unclear, though it has been related to the release of nuclear components following hepatocyte injury and/or to a loss of B cell tolerance to nuclear components.

SMA stains the arterial vessels (V), the mesangium of glomeruli (G) and the fibres surrounding the kidney tubules (T). The VG and VGT patterns have been found to be more specific for AIH than the V pattern alone. The VGT pattern corresponds to the 'F actin' pattern or microfilament pattern, observed in cultured fibroblasts. Neither the VGT or the MF patterns are, however, completely specific for AIH-1 as they are absent in some 20% of AIH-1 patients positive for SMA.

Anti-LKM-1, the serological hallmark of AIH-2, stains the hepatocyte cytoplasm and the distal third of proximal renal tubules. The target of anti-LKM-1 autoantibodies is a 50 kDa

protein, localised in the hepatocytes endoplasmic reticulum and later identified as cytochrome P450IID6 (CYP2D6), an enzyme involved in the metabolism of debrisoquine. Expression of CYP2D6 was subsequently found on the surface of hepatocytes [20], a finding that suggests a direct involvement of LKM-1 autoantibodies in AIH liver damage.

In addition to conventional autoantibodies (ANA, SMA, anti-LKM-1), whose detection is provided by most clinical immunology laboratories, patients with AIH may have other autoantibodies, the presence of which are clinically relevant. These autoantibodies include anti-LC1, anti-perinuclear neutrophil cytoplasm (p-ANCA) and anti-SLA antibodies. Anti-LC1 antibodies can be present alone or in combination with anti-LKM-1 and represent an additional serological marker for AIH-2. Their molecular target has been identified as the formimino transferase cyclodeaminase [21]. p-ANCA can be found in AIH-1 where they react against peripheral nuclear membrane components (hence now preferably termed perinuclear antinuclear neutrophil antibodies, p-ANNA). Anti-SLA, which differently from other autoantibodies are not detected by immunofluorescence, are highly specific for the diagnosis of AIH and their presence is associated with a more severe course of the disease [4, 18]. Their molecular target has been recently identified as the Sep (O-phosphoserine) tRNA-Sec (selenocysteine) tRNA synthase (SEPSEC) [22].

TREATMENT AND CLINICAL COURSE

As soon as the diagnosis is made, treatment should be promptly instituted to obtain complete remission while preventing disease progression. A five-year follow-up study shows that 94% of the patients undergo remission (i.e. normal AST, ALT and IgG and negative or low autoantibody titre) within 2-10 months from starting treatment [12, 16].

Successful treatment is achieved in most cases with inexpensive, well tested drugs. The mode of treatment administration over time is key to success. Treatment of juvenile AIH is initiated

with prednisolone (or prednisone) 2 mg/kg/day (maximum 60 mg/day). This dose should be gradually decreased over a period of 4–8 weeks, guided by the decline of transaminase levels, to a maintenance dose of 2.5-5 mg/day. The target should be an 80% decrease of the transaminase levels by the first two months of treatment: their complete normalization may take several months. During the first 6–8 weeks of treatment, liver function tests should be checked weekly to allow frequent dose adjustments. The attempt to attain normal transaminase levels more rapidly would require a prolonged use of high dose steroids with attendant severe side effects. The timing for the addition of azathioprine as a steroid-sparing agent varies according to the protocols used in different centres. In our centre, azathioprine is added if the transaminase levels stop decreasing on steroid treatment alone, or in the presence of steroid side effects, at a starting dose of 0.5 mg/kg/day, which in the absence of signs of toxicity is increased up to a maximum of 2.0–2.5 mg/kg/day until biochemical control is achieved. In other centres azathioprine is added at a dose of 0.5-2 mg/kg/day in all cases after a few weeks of steroid treatment, when the serum aminotransferase levels begin to decrease [19]. Whatever the protocol, 85% of the patients eventually require the addition of azathioprine to steroids. Some centres use a combination of steroids and azathioprine from the beginning, but caution is recommended because azathioprine can be hepatotoxic, and should be avoided in severely jaundiced patients until the jaundice subsides.

Relapse, characterised by increase in AST and ALT levels, occurs in some 40% of treated patients. It is often related to attempts of drug withdrawal or non-adherence [23], especially in adolescents, and requires an increase in the steroid dose. Long-term immunosuppressive therapy may be occasionally associated with the development of malignancies such as skin cancers and non-Hodgkin lymphomas [24].

With the aim of inducing remission whilst avoiding side-effects associated with high dose steroid treatment, cyclosporine and tacrolimus have been used as steroid-sparing agents [25-

28], but whether these more toxic drugs provide any advantage over standard treatment remains to be tested. Most difficult-to-treat cases respond to mycophenolate mofetil used at 20 mg/kg twice a day in association with predniso(lo)ne [29-32]. Calcineurin inhibitors may have a role in the treatment of those patients who are unresponsive or intolerant to standard therapy (about 10% of cases).

The optimal duration of immunosuppressive treatment for AIH is unknown. Treatment withdrawal is successful only if there is histological resolution of inflammation. Hence, cessation of treatment should be considered if a liver biopsy shows minimal or no inflammatory changes after 1-2 years of normal liver function tests, normal IgG levels and negative or low titre autoantibodies. However, it is advisable not to attempt treatment withdrawal within 3 years of diagnosis or during or immediately before puberty, when relapses are more common. It has been reported that 20% of patients with AIH type 1 can successfully and permanently stop treatment, while this is rarely achieved in AIH type 2 [12]. Long-term treatment is required for the majority of patients, and parents and patients should be counselled accordingly. In the paediatric setting, an important role in monitoring the response to treatment is the measurement of autoantibody titres and IgG levels, the fluctuation of which correlates with disease activity. In particular, for patients with high IgG levels, their decrease is a reliable, objective and inexpensive measure of disease control.

The prognosis of those children with AIH who respond to immunosuppressive treatment is generally good, with most patients surviving long-term with excellent quality of life on low dose medication. Development of end-stage liver disease requiring liver transplantation despite treatment, however, has been reported 8-14 years after diagnosis in 8.5% of children with AIH [12]. Liver transplantation for AIH is successful with 5-year and 10-year patient survival approaching 75%. Recurrence of AIH after liver transplantation has been reported in ~30% of cases at an average time of 4.6 years following transplant. In 6-10% of patients

undergoing liver transplantation for non-autoimmune liver disorders, development of *de novo* AIH has been described, a condition responding to standard treatment for AIH [33], but not to classical anti-rejection therapy. In resistant cases remission has been obtained with rapamycin [34].

A question frequently asked by parents and teen-age girls is the effect of treatment on pregnancy and its safety for the foetus. A few published reports demonstrate that treatment with steroids and azathioprine is safe for the mother and the baby and not associated with an increased risk of foetal defects or mortality [35-37].

AETIOLOGY AND PATHOGENESIS

The aetiology of AIH is unknown, though genetic and environmental factors are involved in its expression.

Genetics: AIH is a complex trait disease, i.e. a condition not inherited in a Mendelian fashion. Susceptibility to the disease is conferred by genes located within the human leukocyte antigen (HLA) region on the short arm of chromosome 6, especially those encoding allelic variants of DRB1. Susceptibility to AIH-1 is conferred by HLA-*DRB1*0301* in adults and children, and *DRB1*0401* in adults among European and North American populations, *DRB1*0405* and *DRB1*0404* in adults in Japan, Argentina and Mexico [38]. Interestingly HLA-*DRB1*1301* has been found to be the AIH predisposing allele in South America, its expression being associated with persistent infection with the endemic hepatitis A virus. This association suggests a potential role for the hepatitis A virus in the pathogenesis of autoimmune liver disease [39]. Susceptibility to AIH-2 is conferred by HLA-*DRB1*0701* and *DRB1*0301*, patients positive for *DRB1*0701* having a more aggressive disease and severe prognosis.

Mechanisms of liver damage: early immunohistochemical studies, focused on the phenotype of inflammatory cells infiltrating the liver parenchyma in AIH, showed a predominance of $\alpha\beta$ T-cells [40] amongst the infiltrating lymphocytes. Most of these cells were represented by CD4 helper/inducer and a minority was constituted of cytotoxic/suppressor lymphocytes. Lymphocytes of non-T-cell lineage were less represented and were composed of NK cells, macrophages and B lymphocytes [40]. Subsequent studies identified IL-2- and IFN γ -producing cells amongst the lymphocytes infiltrating the portal tracts, their number being correlated with histologically assessed disease activity [41].

Regardless of the factors triggering the autoimmune process, the mechanisms leading to, and perpetuating liver damage, act in a complex scenario, which involves the intervention of both cellular and humoral arms of the immune response (Figure 2).

Most of the investigations on the involvement of cellular immune responses in the pathogenesis of autoimmune liver damage have been conducted in the context of AIH-2, whose target autoantigen, CYP2D6, has been identified and widely characterised. A study from Ma and colleagues showed that in HLA-DR7 positive patients, CD4 T-cells recognise seven regions of the CYP2D6 molecule, five of these regions being recognised also by cytotoxic CD8 T-cells. The extent of both CD4 and CD8 T-cell immune responses strongly correlates with biochemical and histological markers of disease activity/severity [42, 43], implicating a participation of both cell types in the pathogenesis of AIH. In addition to CD4 and CD8 T adaptive immune responses, there is evidence that also innate immune mechanisms participate in the autoimmune liver damage. A recent study has shown that compared to health, monocytes, one of the major cell types besides CD4 and CD8 lymphocytes in the hepatic AIH infiltrate, have a more vigorous spontaneous migration, which cannot be further augmented after exposure to chemoattractants [44]. Though the mechanisms underlying the breakdown of immune-tolerance in AIH have not been

completely elucidated, there is now mounting evidence that a defect in immune-regulation plays a key role in leading to immune-tolerance breakdown. An impairment of immune-regulatory mechanisms has been described since the 1980s [45-47]. In more recent years a numerical and functional defect of CD4^{pos}CD25^{pos} regulatory T-cells (T-regs), has been described [48-50]. T-reg numerical defect is more evident at presentation than during drug-induced remission, when a partial T-reg reconstitution is observed, possibly as result of control over inflammatory immune responses exerted by the immunosuppressive drugs. T-reg frequency inversely correlates with markers of disease activity, namely levels of anti-LKM-1 and anti-SLA autoantibody titres, implicating a control of T-regs over the serological manifestations of autoimmune liver disease. With regard to their function, T-regs isolated from children and adolescents with AIH, display defective ability to regulate the proliferation and the effector cytokine production of both CD4 and CD8 responder cells. In addition to impaired regulation of effector T-cell function, T-regs in AIH are unable to restrain, but even promote, the activation of monocytes.

Whether defective immune-regulation in AIH is the result of impaired T-regs only or it is also due to a reduced responsiveness of the effectors to T-reg control, is still unclear. A recent study has shown that T-regs from AIH patients display low levels of Galectin-9, a molecule strictly linked to the ability of these cells to suppress, as it binds to Tim-3, its receptor on effector CD4 T-cells, inducing their apoptosis. Alongside reduced levels of Galectin-9 on T-regs, children with AIH have low expression of Tim-3 on CD4 effector cells, suggesting that defective immune-regulation depends on both impaired T-reg number/function and low susceptibility of effector cells to T-reg control [51].

REGULATORY T-CELLS AS A FUTURE AIH IMMUNOTHERAPY

Despite being defective, T-regs isolated from AIH patients can undergo expansion when exposed to a polyclonal stimulus (i.e. high dose IL-2 and anti-CD3/anti-CD28 T-cell expander). Expanded T-regs express high levels of FOXP3, the T-reg-lineage specific transcription factor, and suppress more efficiently than freshly isolated T-regs [52].

Recently, in the context of AIH-2, we have been able to obtain regulatory T-cells with specificity for CYP2D6, the main autoantigenic target in this condition, and found that these cells suppress much more efficiently than their counterpart generated under non-antigen-specific conditions [53]. CYP2D6-specific T-regs control effectively the proliferation, IFN γ and IL-17 secretion by autoreactive CD4 T-cells and restrain the cytotoxicity of CD8 T-cells [53].

Because of their ability to deliver a tailored form of immunosuppression, i.e. targeted to the autoantigenic regions recognised by autoreactive CD4 and CD8 T-cells, antigen-specific T-regs represent a potential immunotherapeutic tool for restoring immune-tolerance in AIH-2 without inducing pan-immunosuppression.

CONCLUDING REMARKS

We have highlighted epidemiological and clinical aspects alongside serological and histopathological features that may assist in the diagnosis and treatment of juvenile AIH. We have also summarised the immunological mechanisms leading to and perpetuating liver damage in this condition with particular focus on CD4^{pos}CD25^{pos} regulatory T-cells, whose numerical and functional defect is likely to play a role in permitting autoreactive immune responses against liver autoantigens to occur. The generation and expansion of regulatory T-cells in the test tube has opened the possibility to apply these cells in immunotherapy to deliver targeted immunosuppression and possibly immune tolerance restoration.

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Figure Legends

Figure 1 - The portal and periportal inflammatory infiltrate characteristic of autoimmune hepatitis consists of lymphocytes, monocytes/macrophages and plasma cells (interface hepatitis). Haematoxylin & eosin staining. (Picture kindly provided by Dr Alberto Quaglia)

Figure 2 - Autoimmune attack to the liver cell. The autoimmune attack to hepatocytes initiates with the presentation of an autoantigenic peptide to an uncommitted T helper (Th0) lymphocyte. The peptide is embraced by the HLA class II molecule of an antigen-presenting cell (APC). Th0 cells become activated and, according to the nature of the antigen and to the presence in the microenvironment of either interleukin (IL)-12 or IL-4, differentiate into Th1 and Th2 cells. Th1 cells secrete IL-2 and interferon-gamma (IFN- γ) that, in turn, stimulate cytotoxic T-lymphocytes (CTL), enhance expression of class I and induce expression of class II HLA molecules on hepatocytes and activate macrophages; activated macrophages release IL-1 and tumor necrosis factor alpha (TNF- α). Th2 secrete mainly IL-4, IL-10 and IL-13, leading to autoantibody production by B-lymphocytes. If regulatory T-cells (T-reg) do not exert control, a variety of effector mechanisms are triggered: liver cell destruction could derive from the direct action of CTL; cytokines released by Th1 and recruited macrophages; complement activation or engagement of Fc receptor-bearing cells such as natural killer (NK) lymphocytes by the autoantibody bound to the hepatocyte surface. Th17 cells, which arise in the presence of transforming growth factor beta (TGF- β), IL-6 and IL-1 β are also involved in the autoimmune liver attack [54].

Figure 1.

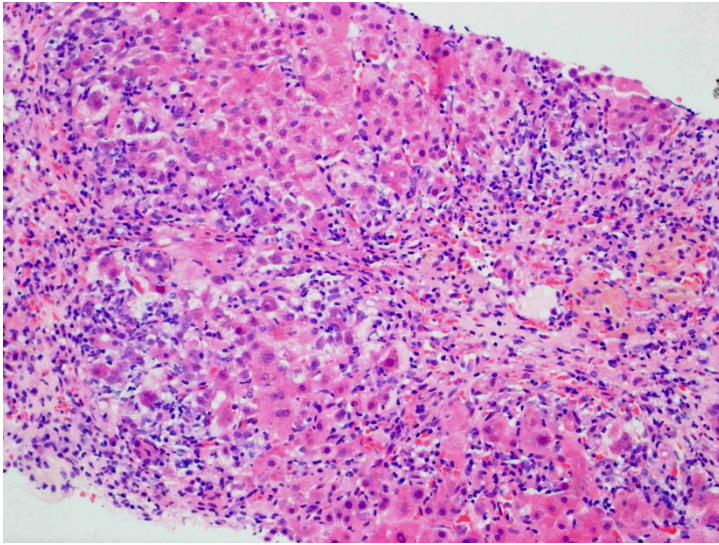


Figure 2

